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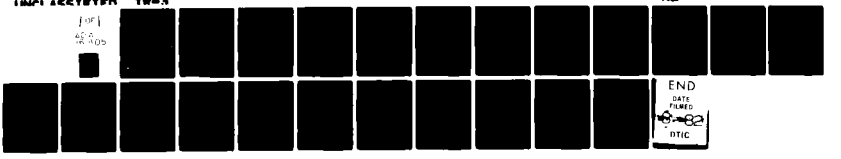

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L. Cunningham and H. Freiser

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Ion Selective Electrodes

for

Basic Drugs

by

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Summary

Coated-wire ion-selective electrodes based on dinonylnaphthalene sulfonic acid (DNNS) are prepared for methadone, methylamphetamine, cocaine, and protriptyline in protonated form. In each set, nearly Nernstian responses are obtained, while detection limits range from $10^{-5.5}$ M for cocaine and methylamphetamine electrodes to $10^{-6.0}$ M for methadone, and $10^{-6.5}$ M for protriptyline electrodes. Selectivity is found to decrease in the order methadone, protriptyline, cocaine, and methylamphetamine; results which are consistent with systematic selectivity studies reported earlier for electrodes in this family.

Introduction

Since the development of DNNS-based poly(vinyl chloride) matrix membrane electrodes as sensors of high-molecular weight cations, electrodes responsive to basic lipophilic drugs (1,2) and to a variety of alkylammonium ions (3) were prepared. In a recent systematic study of selectivity performed in this laboratory (4), it was shown that certain factors in addition to the number of carbon atoms of the cation affect the selectivity displayed by DNNS-based electrodes. These factors included degree of nitrogen substitution, branching of the hydrocarbon chains, and the number and type of hydrophilic substituents; parameters which control the extent to which the analyte (or the interferent) will partition into the PVC membrane.

This paper describes the preparation and evaluation of electrodes selective to methadone, cocaine, protriptyline, and methylamphetamine. These compounds were chosen because they are protonated amines in the physiologic pH range, and are representative of some important families of pharmaceuticals: ~~(Figure 1)~~ methylamphetamine (MW = 150) a central nervous system stimulant; protriptyline (MW = 263) an anti-depressant; methadone (MW = 310) a narcotic analgesic; and cocaine (MW = 303) a local anaesthetic.

-1 p1

Experimental Section

Reagents.

Stock solutions of the drugs were made by dissolving known amounts of the pure hydrochloride salts in 10^{-2} M pH 4.0 acetate buffer. Chromatographic grade Poly(vinyl-chloride) was obtained from Polysciences (Warrington, Pa.) and Dioctylphthalate from Eastman.

Potentiometric Measurement System.

All EMF measurements were made with a previously described Data General Nova 2/10 minicomputer system (4). To allow use of up to five electrodes in sample volumes as small as 1.0 ml, the following cell was constructed. A 3 mm hole was drilled in the bottom of a 10 ml vial, to which a Ag/AgCl electrode made from two Pasteur pipettes was sealed with silicone rubber cement. Polyacrylamide impregnated with 0.1 M KCl and 0.1 M NH_4NO_3 was used for the internal and external junctions, respectively. A 1.000 ml. Mettler digital burette capable of adding 1.0 microliter increments was used for all titrations.

Electrode Preparation and Handling.

Coated-wire electrodes were used exclusively, and were prepared as described previously (4). As before, the electrodes were stored in 10^{-3} M solutions of the species to which they were selective. During the course of calibration and/or

selectivity measurements, the electrodes were kept in buffer solution containing no primary ion. All measurements were made at 25.0 0.1 °C.

Results and Discussion:

Nearly Nernstian responses were obtained for all electrodes with excellent linearity (E vs. log C) over ranges specified in Table 1. The electrodes responded rapidly above a concentration of 10^{-5} M, usually equilibrating within 30 seconds to one minute after exposure to a new concentration. Below this level, however, longer times were required (2 - 5 minutes). For all electrodes, "equilibrium" was assumed when drift was less than 0.4 mV/minute, though at the higher concentrations drift of only 0.1 mV/minute was commonplace.

Selectivity coefficients are reported as $\text{Log}(k^{pot}_{i,j})$ in Table 2. As expected from earlier studies and by inspection of structures for the drugs (Figure 1), selectivity decreased in the order methadone, protriptyline, cocaine, methylamphetamine. The very high selectivity of the methadone and protriptyline electrodes over cocaine and methylamphetamine precluded the precise determination of selectivity coefficients for the smaller amines. In fact, interference from methylamphetamine was so negligible that no changes in EMF readings for methadone and protriptyline electrodes could be attributed to its presence. The concentrations of primary and interfering ion are reported because the selectivity coefficients varied with each of these values.

It was observed that the detection limits obtained depended on the primary ion. This was not apparent from earlier studies,

because primary ions did not vary in molecular weight or structure to the same extent as those used here. The calibration curve shown in Figure 2 for a set of five protriptyline electrodes indicates that detection limits of $10^{-6.5}$ M are obtainable. For methadone, slightly higher detection limits were realized (10^{-6} M), and for the cocaine and methylamphetamine electrodes, detection limits were even higher ($10^{-5.5}$ M).

Calibration curves for the individual electrodes were found to be reasonably reproducible from day to day provided that the electrodes were soaked in 10^{-2} M pH 4.0 acetate buffer between calibrations (Table 1). After exposure to strong interferences, however, the calibration curves shifted by several millivolts so that any further measurements would result in erroneously high results. The original response was restored by storing the electrodes in 10^{-3} M primary ion followed by soaking overnight in the buffer solution. Electrodes prepared five months ago are still functioning normally.

Conclusions

Sternson et. al.(5) reported methadone coated wire electrodes in an earlier paper using PVC impregnated with dioctylphthalate as the membrane material. Though Nernstian responses to methadone with detection limits of $10^{-6.0}$ M along with high selectivity towards inorganic cations was achieved, selectivity among other high molecular weight cations was poor. For DNNS-based electrodes, selectivity increases with analyte lipophilicity. The $k^{pot}_{i,j}$ values obtained for methadone and protriptyline with respect to cocaine and methylamphetamine attest to this. A larger increase in selectivity is seen for protriptyline over cocaine than for methadone over protriptyline, even though the carbon numbers in each pair differ by only 2. This is understandable from the lower distribution constant of cocaine arising from the two carboxylate substituents as well as to the lower degree of substitution of nitrogen in protriptyline. Distribution constants of the drugs in their hydrochloride form for the octanol/water system were calculated by the method of Hansch et al. (6), in which an overall Log K_D value for a compound in a given solvent system is the sum of individual contributions from its molecular fragments. These values increased in the order methylamphetamine (-1.61), cocaine (0.80), methadone (1.37), protriptyline (1.46). Since the contributions (or fragment constants) are derived from experiment, small errors

can arise with these calculations either from unanticipated molecular interactions or to lack of sufficient experimental data for a given fragment. The latter reason is apparently the case for the methadone/protriptyline pair, since the calculated K_D value for protriptyline is slightly higher than that for methadone yet the methadone electrodes displayed greater selectivity. As pointed out by Hansch and Leo (6, p 41) additional data is needed for large fused ring systems bearing a positive nitrogen. Even so, the high selectivity of both of these electrodes over methylamphetamine and cocaine is accurately reflected by the large difference in the distribution constants.

Interference from drug metabolites can also be predicted from their structures. Methylamphetamine does not undergo extensive metabolism and is excreted unchanged (7). Cocaine, however, is rapidly converted to methylecognine through hydrolysis of the benzoyl group (8). This represents a decrease in carbon number of seven, which, based on calculated Log K_D values, indicates that methylecognine will not be a significant interferent (calculated Log K_D = 0.80 for cocaine and -1.64 for methylecognine). Protriptyline is N-demethylated (28) and as inferred by previous work (4), this compound would be expected to be a major interferent. Methadone is also N-demethylated, but then cyclizes to form 2-ethyl-1,5-dimethyl-3,3-diphenyl-1-pyrroline (9). Although this is a quaternary ammonium compound it would probably cause

significant interference as predicted by its its relatively high calculated Log K_D value of 2.13. Preparation of electrodes selective to these interfering metabolites which would not respond significantly to the parent drug would be one approach for monitoring metabolism of these substances. Since both species are often at appreciable levels in urine samples, depending upon the individual drug and others which have been ingested (10), each compound must be analysed simultaneously. With DNNS electrodes, no interference is possible from inorganic cations even at 10,000 fold excess concentration.

Following administration, extremly low levels of drugs are found in serum and urine samples (Table 3). In Table 4, the detection limits of these electrodes are compared to several alternative methods applicable to clinical samples. It should be pointed out that for most of these methods, the reported values were attained only after sample pretreatment methods such as derivitization or preconcentration, whereas those for the potentiometric ISE method involved none of these. With preconcentration, the detection limit of a potentiometric procedure may equal or surpass that of the EMIT technique. The time and cost of an analysis using coated-wire electrodes should be substantially reduced. In addition, the electrodes are more than sufficiently sensitive and reproducible for analysis of pharmaceutical preparations. The drugs studied here represent only a fraction of those for which electrodes may be fabricated. Others may easily be made as needed by adding the compound of

interest as the primary ion in the polymer membrane solution. Selectivity characteristics are then predicted as described earlier or may be determined experimentally if certain interferences are suspected.

It was interesting to note that the electrode of highest selectivity (methadone) did not have the lowest detection limit (protriptyline) . This supports the contention that selectivity is determined primarily by the partition coefficient of the protonated amine, whereas detection limit is determined by solubility of the DNNS-ammonium ion-pair. This suggests that electrodes with lower detection limits could result by using a ligand which can form a more water-insoluble salt with the analyte than can DNNS, or by covalently attaching a ligand to the polymer backbone. Initial studies on ionic polymers in this laboratory (11) have shown promise for this latter approach.

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Literature Cited

1) Martin, C.R.; Freiser, H. Anal. Chem., 1980,52,1772.

2) Yamada, T.; Freiser, H. Anal. Chim. Acta,1981,125,179.

3) Martin, C.R.; Freiser, H. Anal. Chem.,1980,52,562.

4) Cunningham, L.; Freiser, H. Anal. Chim. Acta., in press.

5) Sternson, Larry A.; Higuchi,Takeru; White, Wesley R.; Srianyata, Songsak Anal. Chem.,1978,50(2),232.

6) Hansch, C. and Leo, J.L. "Substituent Constants for Correlation Analysis in Chemistry and Biology", J. Wiley and Sons, New York, 1979, p 40.

7) Clarke, E.G.C. "Isolation and Identification of Drugs", The Pharmaceutical Press, London, 1969.

8) Inaba, T.; Stewart, D.J.; Kalow, W. Clin. Pharmacol. Ther.,1978,23,547.

9) Beckett, A.H., Mitchard, M. and Shihab, A.A. J. Pharm. Pharmacol, 1971, 23, 347.

10) Robinson, Ann. E ; Williams, Faith M. J. Pharm. Pharmacol., 1971,23,353.

11) Martin, C.R.; Freiser, H. Anal. Chem., 1981, 53,902.

12) Javaid, J.I.; Fischman, M.W.; Schuster, C.R.; Dekirmenjian, H.; Davis, J.M. Science,1978,202,227.

13) Van Dyke, C.; Jatlow, P.; Ungerer, J.; Barash, P.G.; Byck, R. Science,1978,200,211.

14) Van Dyke, Craig; Byck, Robert; Barash, Paul G.; Jatlow, Peter Clin. Chem,1977,23,241.

15) Wan, S.H.; Matin, S.B.; Azarnoff, D.F. Clin. Pharm. Ther., 1978,23,585.

16) Hachey, D.L.; Kreck, M.J.; Matson, D.H. J. Pharm. Sci., 1977,66,1579.

17) Uges, D.R.A.; Noten, J.B.G.M. The Serum Concentration of Drugs, Merkus, F.W.H.M., Ed. Excerta Medica, Amsterdam, 1980.

18) Bailey, David N.; Jatlow, Peter J. Clin. Chem., 1976, 22,1697.

19) Biggs, J.T.; Holland, W.H.; Chang, S.; Hipps, P.P.; Sherman, W.R. J. Pharm. Sci.,1976,65,261.

20) Vandemark, F.L.; Adams, R.F.; Schmidt, G.J. Clin. Chem. 1978,24,87.

21) Hubbard, J.W.; Midha, K.K.; Cooper, J.K.; Charette, C. J. Pharm. Sci.,1978,67,1571.

22) Lynn, R.K.; Leger, R.M.; Gordon, W.P.; Olsen, G.D.; Gerber, N. J. Chromatogr.,1977,131,329.

23) McGillard, K.L.; Wilson, J.E.; Olsen, G.D.; Bartos, F. Proc. West. Pharmacol. Soc.,1979,22,463.

24) Mule, S.J.; Bastos, M.L.; Jukovsky, D. Clin. Chem., 1974,20(2),243.

25) von Minden, David L.; D'Amato, N. Anal. Chem., 1977,49,1974.

26) Jindal, Satya P.; Vestergaard, P. J. Pharm. Sci.,1978, 67,811.

27) Jatlow, Peter I.; Van Dyke, Craig; Barash, Paul.; Byck, Robert J. Chromatogr.,1978,152,115.

28) Balkrishena, Kaul; Millian, Stephan J.; Davidow, Bernard J. Pharmacol. Exp. Ther., 1976, 199,171.

29) Campell, D.B. J. Pharm. Phamacol., 1969,21,129.

30) Gal, Joseph Biomed. Mass Spect.,1978,5(1),32.

31) Endo, Masuro; Imicmachi,Hiromi; Moriyasu, Masataka; Hashimoto, Yohei J. Chromatogr., 1980,196,334.

32) Faraj, Bahat A.; Israili, Zafar H.; Kight, Nancy E.; Smissan, Edward E.; Pazdurnik, Thomas J. J. Med. Chem., 1976,19(1),20.

Table 1

Response Characteristics of Coated Wire Electrodes for Basic Drugs

	Slope (mV/Log C)	Y-int (mV)	Linear Range	Detection Limit
Methylamphetamine	58.64 [a] .67 [b] .58	282 [a] 4 [b] 2	10^{-3} – 10^{-5} M	$10^{-5.5}$ M
Cocaine	59.54 [a] .83 [b] .40	504 [a] 5 [b] 2	10^{-3} – 10^{-5} M	$10^{-5.5}$ M
Methadone	58.08 [a] 1.75 [b] .84	398 [a] 22 [b] 4	10^{-3} – 10^{-5} M	$10^{-6.0}$ M
Protriptyline	58.50 [a] .74 [b] .80	475 [a] 11 [b] 3	10^{-3} – 10^{-5} M	$10^{-6.5}$ M

a standard deviation among several electrodes

b standard deviation among individual electrodes

c 10^{-3} M was highest concentration tested

Table 2.

Summary of Selectivity Characteristics for Various

Coated-Wire Ion Selective Electrodes

(Log $k^{pot}_{i,j}$ values)

Electrode:	Methyl-amphetamine	Methadone	Cocaine	Protriptyline
J-ions:				
Methyl-amphetamine:	------	$< -4^{2}$	$-.60^{1}$	$< -4^{2}$
Methadone:	2.41^{1}	------	1.79^{1}	0.40^{1}
Cocaine:	0.54^{1}	-1.96^{1}	------	-1.78^{1}
Protrip-tyline:	2.16^{1}	-0.67^{1}	1.37^{1}	------

1) (primary ion) = (interferent) = 10^{-3} M

2) (primary ion) = 10^{-4} ; (interferent) = 10^{-3} M

Table 3

Concentration of Drugs of Interest in Plasma following Therapeutic Doses

Drug	Dose	Plasma Level	ref.
Cocaine	oral: 2 mg/kg	200 ng/ml ($10^{-6.2}$ M)	12,13
	nasal: 15-150 mg	2-25 ug/ml ($1\text{-}8\times10^{-5}$ M)	14
Methyl-amphetamine	10 mg	20 ng/ml (1.3×10^{-7} M)	15
Methadone	1 mg/kg-day	100-400 ng/ml (3×10^{-7} M)	16
Protriptyline	2-3 mg/kg-day	50-200 ng/ml (2×10^{-7} M)	17

Table 4

Detection Limits for Various Assay Procedures

for Serum and Urine

(reported as ng/ml)

	Protriptyline	Methadone	Cocaine	Methylamphetamine
GLC	10[i] (18)	5[a] (22)	200[a,h] (25)	2 (29)
GC-MS	5 (19)	5 (16)	2 (26)	0.5 (30)
HPLC	10[g] (20)	---	100 (27)	2[e,b] (31)
RIA	10[f] (21)	1[d] (23)	2 (28)	0.5 (32)
EMIT	---	500(24)	1000 (24)	2000(24)
ISE[c]	85	350	1000	500

a- FID used

b- UV detector used

c- all 1 ml samples, no preconcentration

d- stereospecific for d and l isomers

e- preceeded by derivitization with B-Napthoquinone-4-sulfonate

f- poor specificity with respect to other tricyclic anti-depressants

g- protriptyline was internal standard for analysis of other tricyclic anti-depressants

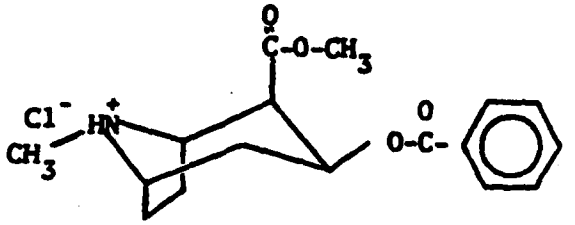
h- cocaine and metabolites extracted, propylated, and back-extracted

i- Nitrogen detector used

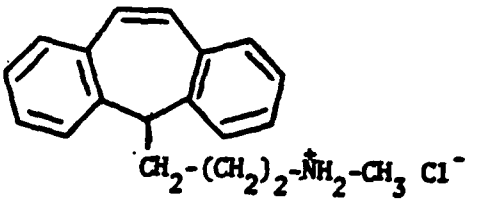
Legends for figures

Figure 1. Structures of basic drugs

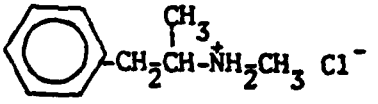
Figure 2. (Potential vs. Ag/AgCl) vs. Log (protriptyline) for five different protriptyline electrodes.



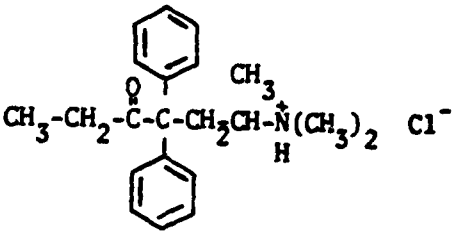
Cocaine HCl



Protriptyline HCl

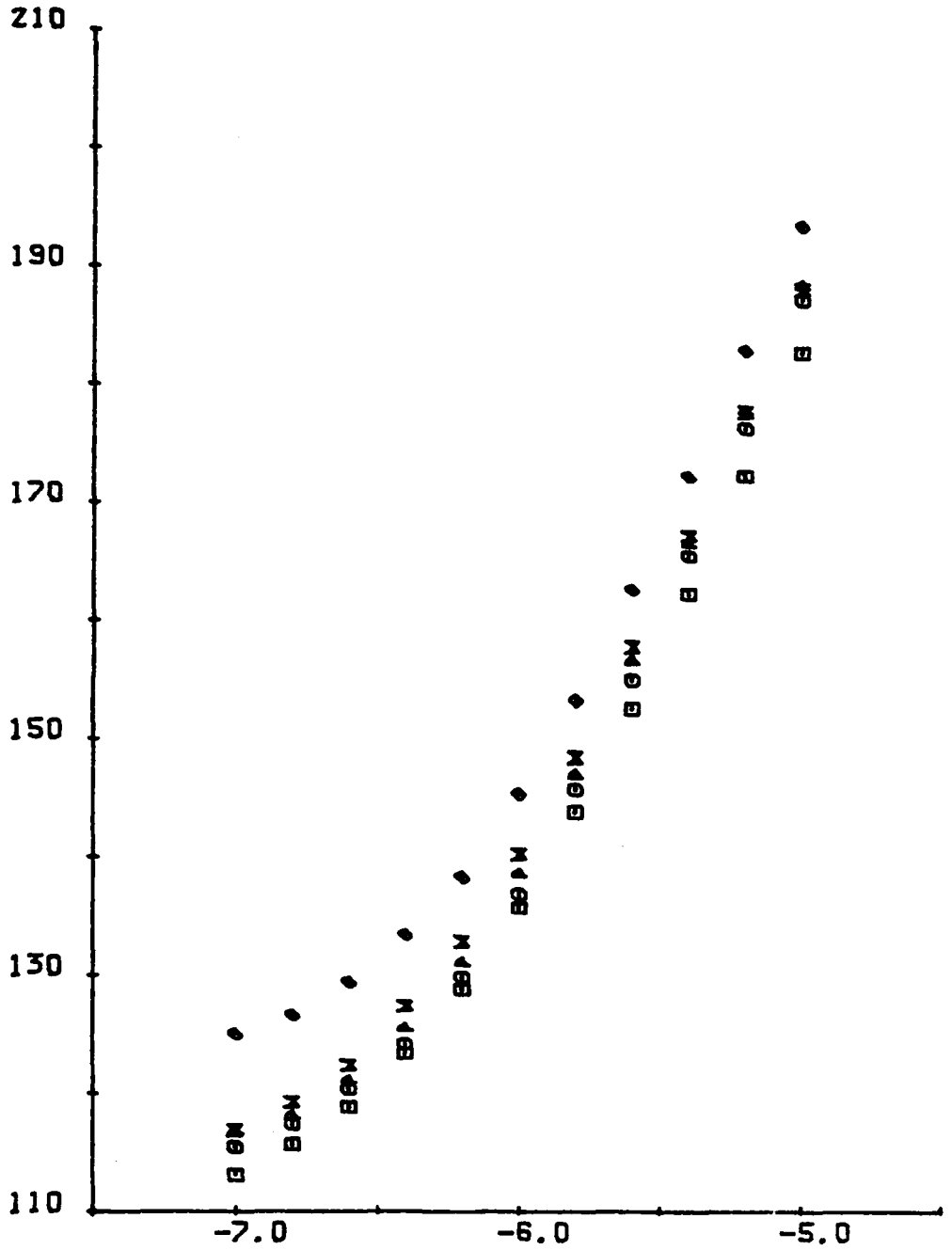


Methylamphetamine HCl



Methadone HCl

Fig. 1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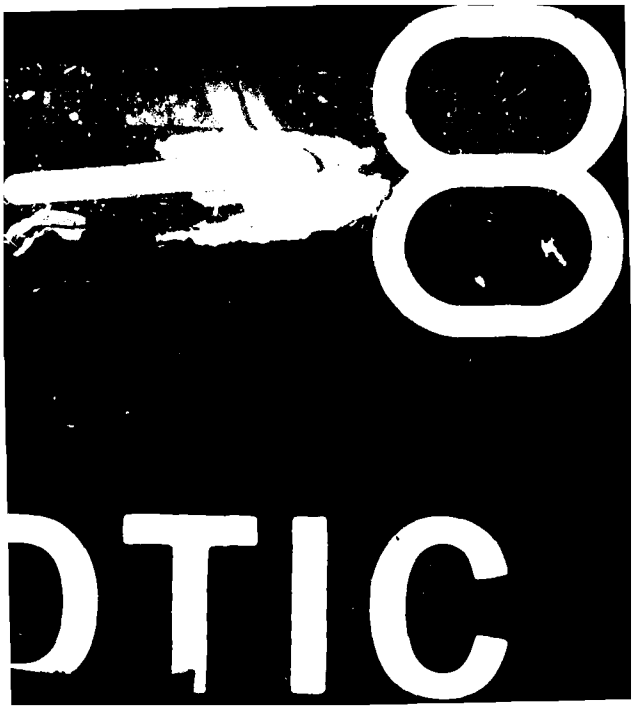
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